FAT METABOLISM OF THE CHICK EMBRYO UNDER STANDARD CONDITIONS OF ARTIFICIAL INCUBATION

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The bird's egg consists of about forty per cent of fat. The largest portion of the fat is found in the egg yolk. In addition to this, during incubation period some fat is synthesized from protein and some possibly from carbohydrate molecules. All this fat is utilized for nutrition and energy supply of the developing embryo and of the young bird after hatching.

Among the earliest workers with avian eggs Parke (1866) noticed that the amount of fat in the egg yolk diminishes during incubation. Then Eaves (1910), Sakuragi (1917), Idzumi (1924) and Murray (1926) showed experimentally that as the actual amount of fat decreases in a whole egg, it increases in an embryo. Needham (1925 and 1927) in his reviews of the literature pointed out that the fat in an egg is the most important energy-source of the developing embryo.

The above facts would suggest that the egg fat is of such chemical composition that it can be utilized according to the need of the embryo. The process of absorption of fat, particularly of yolk fat, also gives another aspect of the question. It looks very much as if there is a preferential absorption of the unsaturated acids by the embryo at certain stages of incubation (Eaves, 1910).

The present paper consists of a further experimental study of the fat metabolism of the chick embryo under standardized or "normal" conditions of artificial incubation, that is, changes of the amount and chemical nature of fat in the embryo and egg yolk as measured by the iodine value, saponification number and refractive index.

Methods and Materials

All eggs used were from a flock of White Leghorn hens (*Gallus domesticus*). The eggs were selected for uniformity of size and quality of eggshell (Romanoff, 1929).

The incubation was carried on in a special electric laboratory incubator (Romanoff, 1929*a*) under the conditions already described (Romanoff, 1930) with the temperature 38.0 ± 0.2° C., and the relative humidity 60.0 ± 1.0 per cent.

TABLE I

Iodine Value, Saponification Number and Refractive Index of Fat (ether extract) of Yolk of Fowl's Egg

Author	Percentage of Fat in Wet Weight	Iodine Value	Saponification Number	Refractive Index
	per cent			$(n)_D$
Parke (1866)	22.82	—	—	—
Amthor and Zink (1897)	—	75.4	189.4	1.4670
Kitt (1897)	19.00	71.1	190.2	—
Peninngton (1909)	32.39	63.6	182.2	1.4611
Serono and Pallazi (1911)	20.17	82.31	198.85	—
Thomson and Sorley (1924)	31.10	74.73	183.79	—
Romanoff (present rpt.)	30.71	73.89	190.69	1.4690

At intervals of 24 hours all eggs were candled for dead embryos, and at least four eggs with normally developed embryos were removed for analysis.

Previous experience in our laboratory showed that at certain stages of embryonic development it is almost impossible to separate the yolk from albumen or allantoic fluid, and to determine it quantitatively with appreciable accuracy. Therefore the boiling of eggs,—to complete coagulation of yolk and albumen,—had been employed. This method proved to be very quick and satisfactory, not only in determining the total values of yolk and albumen, but also in some chemical analysis, such as fat (ether extract) determination.

TABLE II

Distribution of Fat (ether extract) in a Fresh and Hatched Egg

Parts of the Egg	Fat (ether extract)	
	Amount	Percentage
	grams	*per cent*
Fresh egg:		
Yolk	5.8275 ± 1.0971	99.50
Albumen	0.0046 ± 0.0013	0.08
Shell	0.0033 ± 0.0025	0.06
Shell membranes	0.0213 ± 0.0167	0.36
Total	5.8567	100.00
Hatched egg:		
Chick	1.8887 ± 0.1224	48.57
Yolk sac	1.9315 ± 0.0762	49.67
Shell with waste matter	0.0682 ± 0.0126	1.76
Total	3.8884	100.00
Difference (combusted fat)	1.9683	33.61

TABLE III

Changes in the Fat (ether extract) Content of the Chick Embryo

Stage of Incubation	Weight of Embryo			Fat (ether extract) Content			
	Wet Weight	Dry Weight	Percentage of Dry Matter	Composed Samples	Average Fat Content	Percentage of Wet Weight	Percentage of Dry Weight
days	*grams*	*grams*	*per cent*	*embryos*	*grams*	*per cent*	*per cent*
8	1.350	0.099	7.33	18	0.0133	0.99	13.13
9	2.083	0.147	7.06	12	0.0233	1.12	15.85
10	2.700	0.238	8.81	6	0.0310	1.15	13.03
11	3.850	0.305	7.92	6	0.0539	1.40	17.67
12	5.499	0.513	9.33	5	0.1015	1.85	19.79
13	7.213	0.703	9.75	4	0.1610	2.22	22.90
14	9.670	1.159	11.99	2	0.2575	2.66	22.22
15	11.327	1.544	13.63	2	0.3475	3.07	22.51
16	15.360	2.582	16.81	2	0.5012	3.26	19.41
17	18.095	3.396	18.77	2	0.7997	4.42	23.55
18	22.310	4.220	19.92	2	0.9823	4.40	23.28
19	29.891	5.812	19.44	1	1.3024	4.36	22.41
20	31.298	5.999	19.17	1	1.4053	4.49	23.43

TABLE IV

Changes in the Fat (ether extract) Content of the Yolk Sac

Stage of Incubation	Average Egg Weight	Weight of Yolk Sac			Fat (ether extract) Content		
		Wet Weight	Dry Weight	Percentage of Dry Matter	Average Content	Percentage of Wet Weight	Percentage of Dry Weight
days	*grams*	*grams*	*grams*	*per cent*	*grams*	*per cent*	*per cent*
0	61.6	18.44	9.654	52.35	5.6633	30.71	58.66
1	62.0	19.19	10.110	52.68	6.4837	33.79	64.17
2	62.5	19.79	10.113	51.10	6.0309	30.47	59.54
3	62.5	20.10	9.546	47.49	6.0338	30.02	63.21
4	62.5	21.79	10.390	47.68	6.5621	30.12	63.16
5	61.3	20.22	8.781	43.43	5.3549	26.48	60.98
6	61.8	21.51	8.872	41.24	5.4276	25.23	61.18
7	62.3	21.81	9.354	42.88	5.8198	26.68	62.22
8	62.5	20.58	8.611	41.84	5.4874	26.66	63.73
9	60.3	19.84	8.726	43.98	5.6041	28.25	64.22
10	62.0	19.08	8.767	45.95	5.2271	27.39	59.62
11	61.3	18.80	8.382	44.59	5.3328	28.37	63.62
12	61.5	18.27	8.617	47.17	5.4158	29.64	62.85
13	62.0	18.31	9.097	49.68	5.4335	29.68	59.72
14	59.3	16.64	9.539	57.35	5.5897	33.59	58.59
15	62.5	16.34	9.676	59.25	5.4803	33.55	56.64
16	60.0	14.54	9.006	61.94	4.5241	31.11	50.23
17	61.0	14.02	9.132	65.14	4.4922	32.04	49.19
18	62.5	13.87	8.255	59.52	3.0893	22.27	37.42
19	59.5	12.03	6.986	58.57	2.4567	20.42	35.17
20	60.5	10.53	6.015	57.12	2.1434	20.35	35.64

In these experiments two eggs were boiled for 20 minutes, the coagulated yolk was weighed, then dried to constant weight in a Freas electric vacuum oven at 55° C. and vacuum at about 63.5 cm., ground and subjected for 48 hours to extraction of fat with anhydrous ether by a Soxhlet extraction apparatus.

From two or more unboiled eggs the embryos were separated for determination of dry matter and fat content by methods similar to those described above.

The extracted fat from both the embryo and the yolk sac was tested for saponification number (Koettstorfer, 1879), iodine value (Wijs, 1898),[1] and refractive index, by a Zeiss butter refractometer.

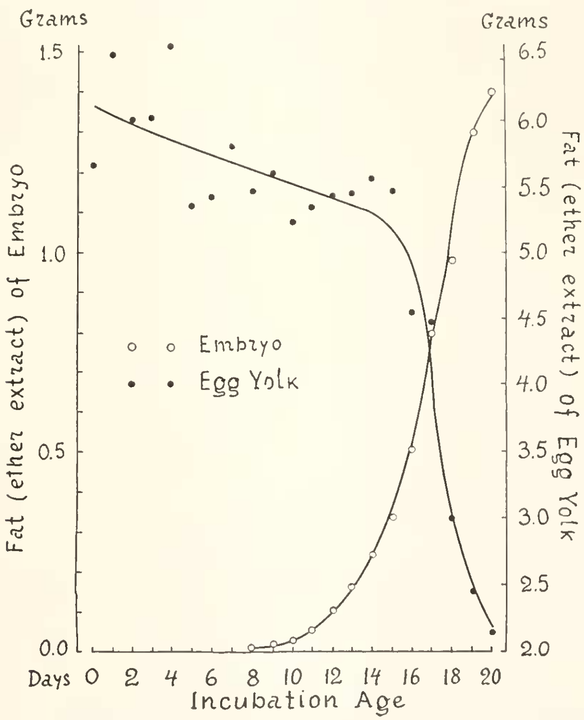

FIG. 1. Changes in the fat (ether extract) content of the embryo and egg yolk during incubation.

Results and Discussions

It has been found that the fat content of a fresh fowl's egg is directly proportional to its size. For that reason the amounts of fat obtained from an egg by previous workers can hardly be compared,

[1] Both saponification number and iodine value were determined after the methods given in *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists*, third edition, 1930, on pp. 321–322.

unless the data are expressed in percentages of dry or wet weight of the substance. Similar inconsistency is observed in the data on iodine value, saponification number and refractive index of the fat from an egg yolk (Table I). These dissimilarities might be accounted for by the lack of uniformity in material and methods, such as: the method of extraction, care of the extract, age of eggs and possibly seasonal quality of eggs.

It is evident from the data of Table II that the egg yolk is the main source of fat to the developing embryo. In a fresh egg there is

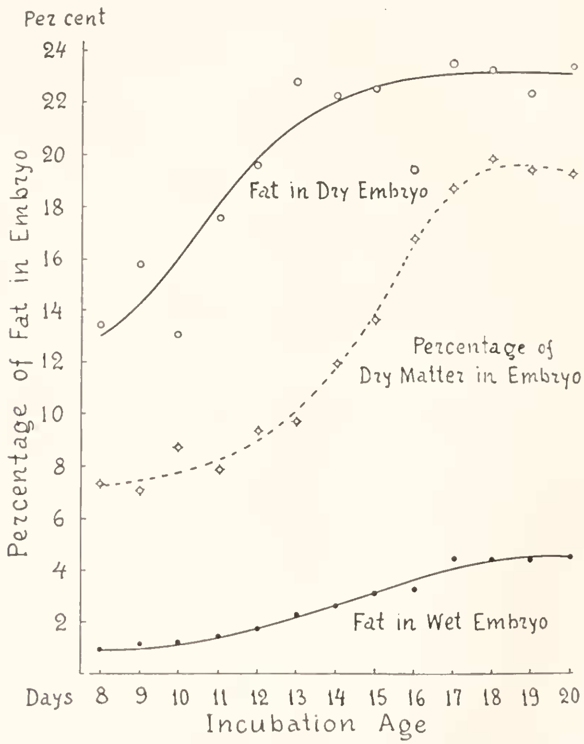

FIG. 2. Percentages of fat (ether extract) in the wet and dry weights of the embryo at various stages of incubation. (The curves of the above values are not identical due to changes in the dry matter content of the embryo, which is shown by dotted line.)

about 99.5 per cent of yolk fat and only 0.5 per cent of fat in the remainder of the egg, including albumen and shell with shell membranes. At the time of hatching the yolk sac still has the largest portion of reserve fat for the nutrition of the chick after hatching. Next in amount is the fat absorbed by the embryo. Then a small amount of fat is retained in the waste matter and in the shell with shell membranes. The combusted fat represents 33.61 per cent, or perhaps a larger amount if synthesis of fat had taken place during the incubation.

The daily fat content of the developing embryo and egg yolk (Tables III and IV) gives a general idea of the changes which go on within an egg during incubation (Fig. 1). The fat of the embryo increases noticeably only after about two weeks. The fat of the yolk at first decreases slowly; then it rapidly falls from about the sixteenth day up to hatching time.

The curves on the percentages of fat in the wet and dry embryo (Fig. 2) are not identical. Change in the moisture content of the embryo throughout the incubation period is the main influencing factor. Therefore a true percentage value should be taken from dry rather than wet weight of the embryo.

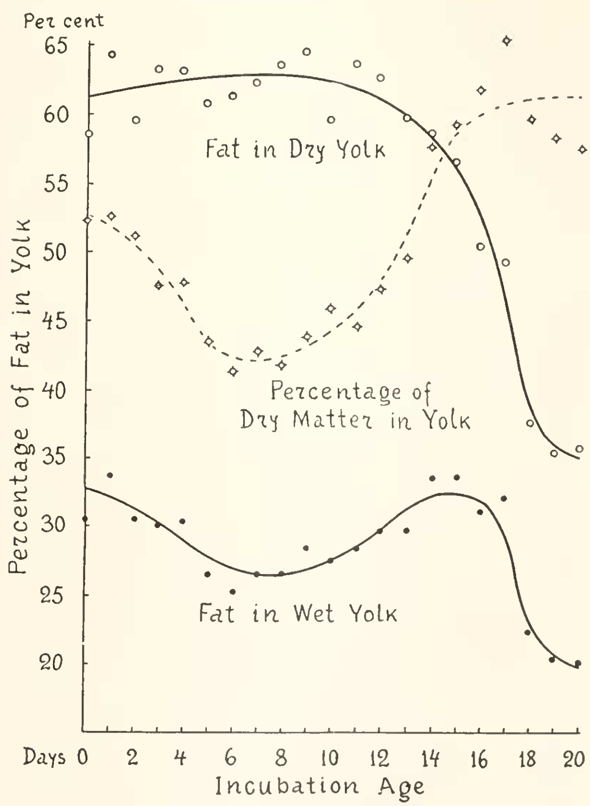

FIG. 3. Percentages of fat (ether extract) in the wet and dry weights of the egg yolk at various stages of incubation. (The curves of the above values are not identical due to changes in the dry matter content of the yolk, which is shown by dotted line.)

The influence of the moisture content of the egg yolk on the curves of the percentages of fat from wet and dry weight (Fig. 3) is still greater than the influence of the moisture content of the embryo. The curve of the percentage of fat in dry yolk is the only one which demonstrates regularity in changes of fat during incubation. This curve shows that the percentage of fat in yolk is increasing somewhat during the first week, declining during the second and dropping during the third week. The rise of the curve may be due to synthesis of fat from other chemical substances; the decline due to the noticeable growth of the embryo; and the drop due to the rapid and sole utilization of fat for growth and for energy supply to the embryo.

TABLE V

Refractive Index of Fat from the Chick Embryo and Yolk Sac

Stage of Incubation	Refractive Index $[(n)_D]$	
	Embryo Fat	Yolk Fat
days		
0	—	1.4685
9	1.4879	—
10	[1.4903]	—
11	1.4877	—
12	1.4841	—
13	1.4844	1.4687
14	1.4816	1.4695
15	1.4758	1.4693
16	1.4763	1.4695
17	1.4741	1.4675
18	1.4735	1.4691
19	1.4727	1.4696
20	1.4719	1.4690

The combusted fat accompanying embryonic development, is the expression of the energy expended. It has been observed that the greatest part of the egg fat is used toward the end of hatching time. The curve of the combusted fat would be very similar to the curve of the growth of the embryo (Romanoff, 1930).

The extracted fat from egg yolk throughout the incubation period shows hardly any change in the saponification and iodine numbers and refractive index, giving on an average 73.89, 190.69, and 1.4690 respectively. On the other hand, there was found a pronounced change in the iodine number and refractive index of the fat from the embryo (Table V, Fig. 4). The iodine number of the fat from the embryo at 16 days of incubation was 78.84; on the following days it

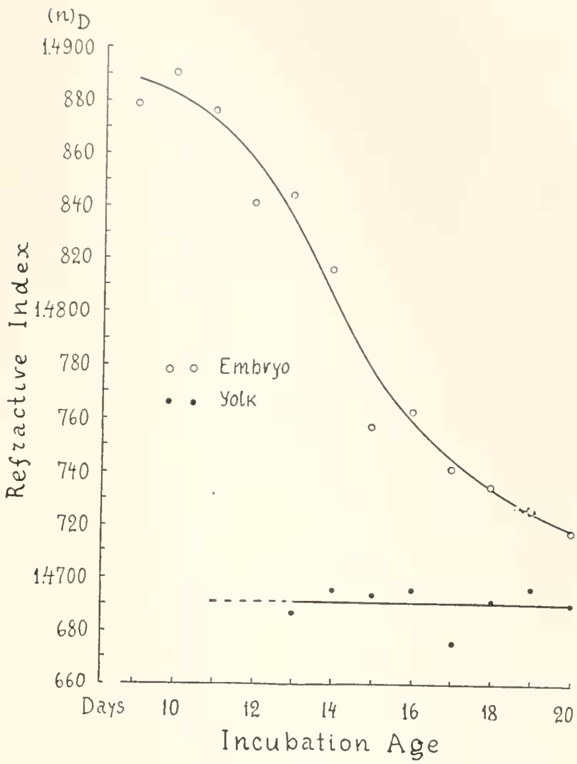

FIG. 4. Refractive index of the fat from the embryo and egg yolk during incubation.

was 82.03, 83.69, 84.03, 88.20, reaching 90.34 at the time of hatching. The refractive index was found to be high at the beginning of observation, nine days of embryonic age, and was rapidly decreasing towards hatching.

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SUMMARY

1. Fat content of fresh eggs is a subject of great variation, due primarily to disparity in size of individual eggs.

2. Iodine value, saponification number and refractive index of the fat from fresh eggs are rather constant, but only under a given experimental condition.

3. The main source of fat in an egg is the egg yolk, which furnishes the fat for nutrition and energy-supply of the developing embryo.

4. The amounts of fat in the growing embryo and egg yolk give a comprehensive idea about the changes in fat which go on within an egg during incubation.

5. The relative increase of fat in the embryo and the decrease of it in the yolk can be well demonstrated by the curves plotted from the data on the percentages of fat in dry weight.

6. The curve of combusted fat is quite similar to the curve of the growth of embryo.

7. Iodine value, saponification number and refractive index of the fat from the yolk sac of the developing egg are almost constant through the incubation period.

8. Iodine value and refractive index of the fat from the developing embryo are increasing and decreasing respectively during the latter part of incubation.

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